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The Design and Synthesis of Inhibitors of Imidazoleglycerol Phosphate Dehydratase as Potential Herbicides*

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Abstract: The discovery and exploration of a new class of broad-spectrum post-emergent herbicides with a novel mode of action, namely the disruption of histidine biosynthesis *via* inhibition of imidazoleglycerol phosphate dehydratase (IGPD; E.C. 4.2.1.19), are described. Clear structural similarities between the first member, produced during an attempt to produce analogues of glyphosate, and intermediates in histidine biosynthesis were noted. Several series of targets were designed, to mimic both the enzyme substrate and a putative reaction intermediate, and synthesised. Some examples were found to be both extremely potent inhibitors of the enzyme, having K_i values as low as 0·3 nM, and phloem-mobile herbicides with levels of weed control similar to those shown by glyphosate. Further logical progress awaits a high-resolution X-ray structure determination of an enzyme-inhibitor complex.

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Key words: triazole, phosphonate, post-emergent herbicides, histidine biosynthesis, imidazoleglycerol phosphate dehydratase, IGPD, inhibition

1 INTRODUCTION

It is now well established that many of the most successful commercial herbicides are active *via* the inhibition of essential amino acid biosynthesis.[1,2] Phosphonates can provide not only metabolically stable mimics of phosphate enzyme intermediates[3] but also the phloem mobility essential for adequate post-emergent control of regrowth in perennial weed species. Useful herbicidal activity of inhibitors of aromatic amino acid biosynthesis was, in 1981, limited to glyphosate **1** and a very few close analogues, and indeed still is. At that time, there was literature speculation that the mode of action might involve chelation with cations, especially those of transition metals.[4] Furthermore, the propensity of 1,2,4-triazoles to bind to iron, for example, was already well-known in fungicides inhibiting the cytochrome P_{450} enzyme, lanosterol 14-α-demethylase.[5]

The triazolylphosphonate **2** (Fig. 1) was designed to have potential binding sites (asterisked) superimposable upon those of **1.** Made simply as shown in Fig. 2, this did indeed prove to have levels of broad-spectrum phloem-mobile herbicidal activity similar to those of glyphosate.[6] However, unlike **1**, it did not inhibit 5-enolpyruvylshikimate 3-phosphate synthase (E.C. 2.5.1.19) and, indeed, did not otherwise affect aromatic

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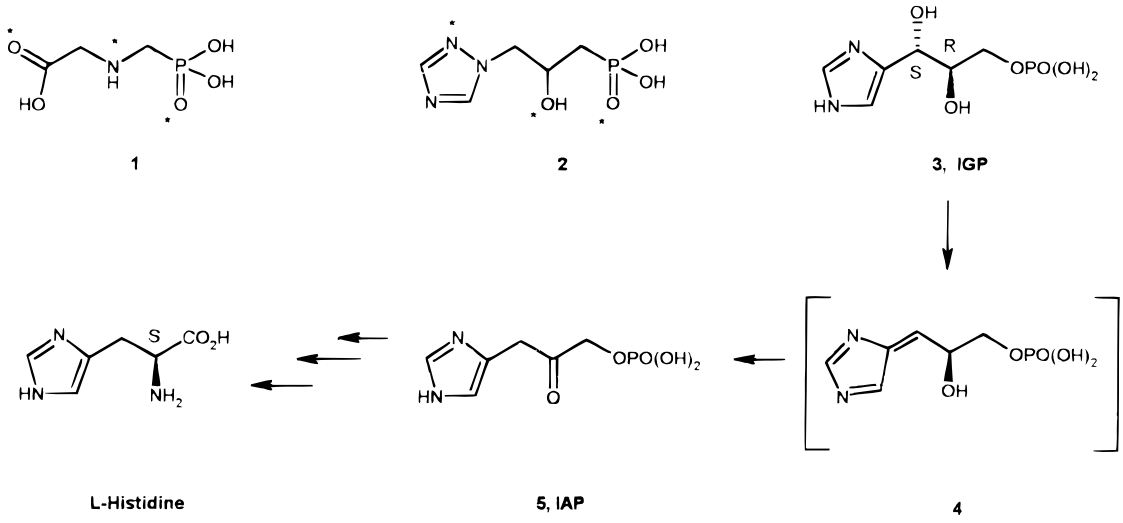


Fig. 1. Comparison of the structures of a novel herbicide **2** with both intermediates in the histidine biosynthetic pathway and the known herbicide, glyphosate, **1**: Asterisks in **1** and **2** indicate superimposable binding sites (see text).

amino acid biosynthesis. There are, moreover, clear structural similarities between **2** and intermediates in histidine biosynthesis, in particular (2*R*,3*S*)-imidazoleglycerol phosphate (**3**; IGP) and the subsequent acetol phosphate (**5**; IAP), the conversion being catalysed by imidazoleglycerol phosphate dehydratase (IGPD, E.C. 4.2.1.19). It was rapidly demonstrated that the effects of **2** could be reversed by application of histidine, both in maize-cell culture and also in certain whole plants, for example *Cynodon dactylon* (L.) Pers. (Bermuda grass). Furthermore, it was also a very active inhibitor of IGPD isolated from *Saccharomyces cerevisiae* Meyer ex Hansen.[7] Whilst then little studied,[8–11] this enzyme is now of considerable interest both in

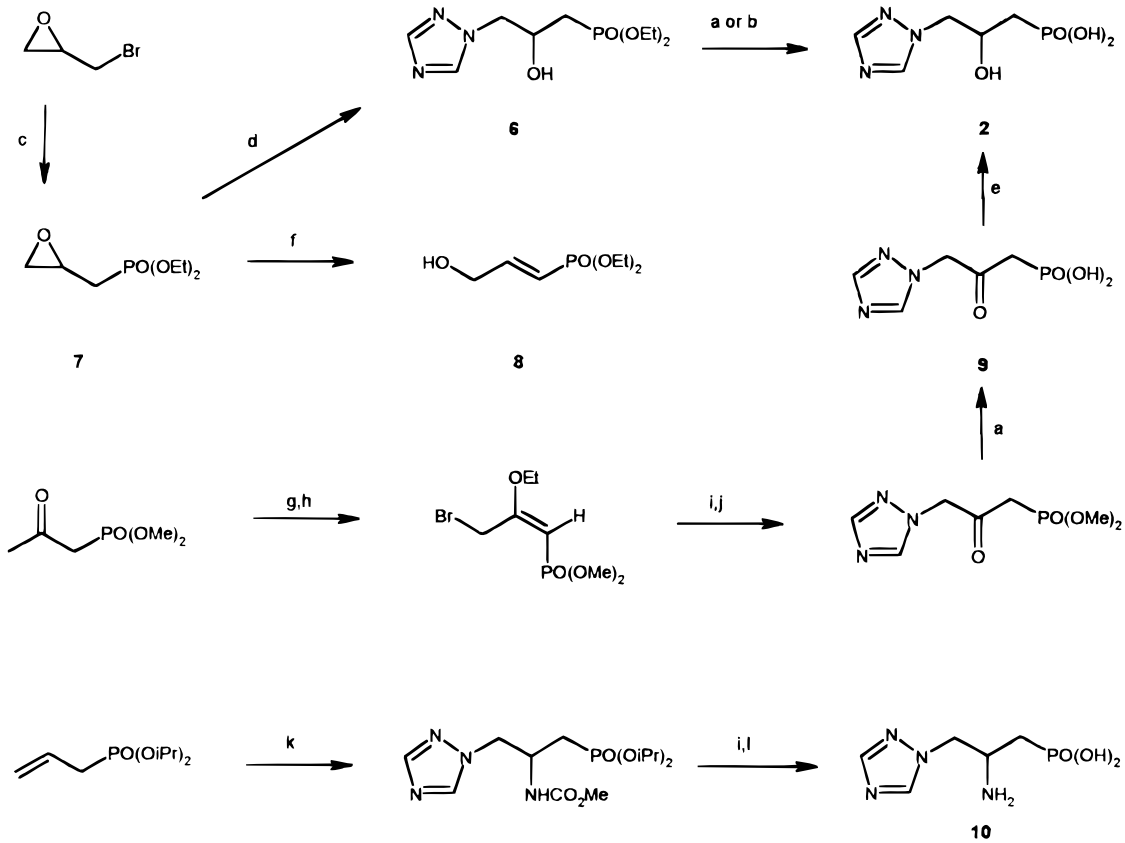


Fig. 2. Syntheses of the *N*-linked triazolylphosphonates, compounds **2** and **10**. (a) Me_3SiBr, CH_2Cl_2 ; H_2O; Amberlite CG-120 (b) 12 M HCl, 100° (c) $P(OEt)_3$ (d) 1,2,4-Triazole; CsF, MeCN or K_2CO_3, MeOH (e) Excess $NaBH_4$, MeOH (f) K_2CO_3, MeCOEt (g) $HC(OEt)_3$, $FeCl_3.6H_2O$ (h) *N*-Bromosuccinimide, CCl_4, hν (i) 1,2,4-Triazole, NaH, THF (j) 12 M HCl, 20°, 10 min (k) AgCNO, I_2, CH_2Cl_2 ; MeOH (l) Me_3SiI, CCl_4 ; H_2O.

TABLE 1
Inhibition Constants for Simple Heterocycles against IGPD

Compound	K_i (μM)	*Compound*	K_i (μM)	*Compound*	K_i (μM)
1,2,4-Triazole	83	Pyrazole	$\geqq$9500	2-Amino-1,3,4-thiadiazole	305
3-Amino-1,2,4-triazole	10	1,2,3-Triazole	$\geqq$3000	5-Amino-1,2,4-thiadiazole	160
1,2,4-Triazolin-3-one	320	Tetrazole	130	2-Amino-pyrazine	1140
Imidazole	1360	Thiazole	2860	2-Amino-pyrimidine	$\geqq$1250

respect of its structure[12–14] and mechanism of action[7,15–20] and also the design of inhibitors as potential herbicides.[6,7,17,18,20–27] Purification and characterisation of the IGPD of *S. cerevisiae* from recombinant *Escherichia coli* (Mig.) Cast. & Chalm. allowed development of a novel continuous assay.[7,12] A monofunctional dehydratase has also been isolated from wheat germ and characterised,[14] whilst mechanistic studies have utilised a bacterial enzyme.[15,16] Coincidentally the enzyme does, in fact, require a transition metal, notably manganese, although this is not directly involved in binding at the active site.[7,12] Racemic **2** was shown to be an extremely potent (K_i 0·6 nM at pH 7·0) and slow-dissociating (K_{off} 0·04 min^{-1}) inhibitor, the K_m for the substrate **3** (Fig. 1) being 105 μM.[7]

2 EXPERIMENTAL METHODS

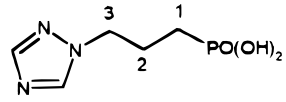
Synthetic methods are outlined in Figs 2–14. Details and spectroscopic data for the majority of compounds described have been published previously.[6,21,22] Spectroscopic evidence for the remainder (e.g. **10**–**12**, **14**, **35**–**37**, and products in Fig. 4) is consistent with the structures shown. Enzyme assays[7,12] and herbicide testing[6,21,22] were performed according to published procedures. K_i values are shown in Tables 1 and 2. Since measurement of weed control was complicated both by seasonal variation and an unusually flat dose response, a detailed table of herbicide results would not be meaningful. However, comparisons between specific pairs of compounds, as discussed in the text, are based on data from single tests and are thus valid.

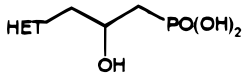
3 RESULTS AND DISCUSSIONS

Whilst phytotoxicity of the herbicide amitrole (3-amino-1,2,4-triazole) has been ascribed primarily to effects upon carotenoid biosynthesis,[28,29] it was already known both to inhibit IGPD and to be synergised by added phosphate.[8–11] Could both the relative spatial disposition of heterocycle and phosphorus residues be optimised and additional binding groups on the linkage be provided to mimic, more effectively, either the substrate **3** or, preferably, the putative[7] diazafulvene reaction intermediate **4** (Fig. 1)? Assays were performed on a wide variety of monocyclic heterocycles, and their amino- and hydroxy-substituted derivatives. Results for a selection are shown in Table 1. A number were incorporated into target molecules to establish whether the relationship still held; in particular, was the nature and direction of the nitrogen lone pairs important? Whilst subject to nucleophilic attack at the less hindered methylene group, the epoxyphosphonate **7** (Fig. 2) is also prone to base-catalysed rearrangement, giving the allylic alcohol **8**. Thus, whereas treatment of **7** with 1,2,4-triazole in the presence of potassium carbonate gave the desired triazolylalcohol **6**, a similar reaction with pyrazole gave only **8**. Caesium fluoride was found to be an excellent substitute base, markedly reducing rearrangement. Imidazole required no added base. 1,2,3-Triazole and tetrazole gave primarily 1-alkylated material. As expected from the K_i values for simple heterocycles, as shown in Table 1, the second most active phosphonic acid of this subset was the tetrazole (K_i 900 nM), more than three orders of magnitude less active than **2**. Electronic similarity calculations shown that **2**, and the proposed corresponding C-linked target **24** (Fig. 6), mimicked the intermediate **4**, rather than the substrate **3** itself. In contrast, the analogous imidazoles were quite unlike **4**.

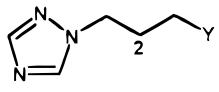
Using similar extended conformations, the distance between the heterocyclic and phosphonate moieties in the inhibitor **2** is less than that in the substrate **3**, as viewed on a 3D graphics screen and measured using 'Viking', an ICI in-house program. Analogues (**11**–**14**), containing either one additional methylene unit or an oxygen atom, were prepared as shown in Fig. 3. The metabolically labile phosphate **11** is a potent inhibitor (K_i 10 nM) but, not surprisingly, it is essentially inactive *in vivo*. The phosphonates **12–14** (K_i 470 nM, 750 nM and 7 μM, respectively) are considerably weaker inhibitors and of no interest. It has been postulated that the already shortened **4** may exist in a folded conformation in which the heterocycle and phosphate groups are very much closer together.[7] This would allow the phosphate dianion to act as an internal base to remove the C-2 hydrogen atom, as previously proposed[30] for dehydroquinate synthase. An improved fit of those inhibitors containing only a three-carbon linkage would then be expected. Recent work, describing compounds in which a five-atom linkage has been introduced, has suggested that these may be able to fold into conformations akin

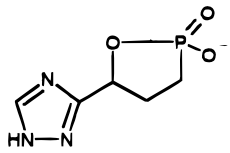
TABLE 2
Inhibition Constants for Potential Herbicides against IGPD



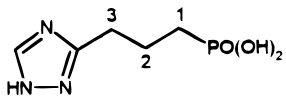


Substituent	*Compound No.*	K_i (μM)	*Substituent*	*Compound No.*	K_i (μM)
2-OH	**2**	0·0006	Imidazol-1-yl	—	2·9
None	—	0·072	Pyrazol-1-yl	—	27
2=O	**9**	1·0	1,2,3-Triazol-1-yl	—	8·3
2-F	—	0·031	Tetrazol-1-yl	—	0·9
2-NH_2	**10**	0·015			
2-CH_2OH	**13**	0·75			
1,1-F_2, 2-OH	**65**	0·008			





Substituent	*Compound No.*	K_i (μM)	*Substituent*	*Compound No.*	K_i (μM)
2-OH, Y = PO(Me)OH	—	2·24	None	**56**	[a] > 0·5
2-OH, Y = PO(OEt)OH	—	13			
2-OH, Y = $OPO(OH)_2$	**11**	0·01			
2-OH, Y = SO_3H	—	9·5			
2-OH, Y = CO_2H	—	4·5			
Y = $B(OH)_2$	—	0·89			

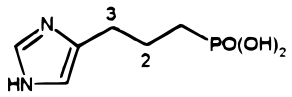


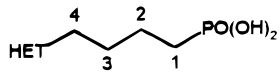
Substituent	*Compound No.*	K_i (μM)	*Substituent*	*Compound No.*	K_i (μM)
None	—	9·6	3-Br	—	[a]
3-OH	**19**	0·011	3-NH_2	**53**	0·0095
2-OH	**24**	<0·001	3(1,2,4-Triazol-1-yl)	—	3·6
syn 2,3-$(OH)_2$	**28**	0·0018	3-I	—	[a]
anti 2,3-$(OH)_2$	**30**	0·253	3-ONO	—	[a]
anti 2-OH, 3-NH_2	**39**	0·075	3-OCOMe	—	0·095
syn 2-OH, 3-NH_2	**41**	0·008	3-NO_2	—	0·8
anti : *syn* (9 : 1)2-NH_2, 3-OH	**45** : **46**	0·58	2-NH_2	**52**	0·024
anti 2,3-$(NH_2)_2$	**47**	0·58	*syn* 2,3-$(OH)_2$, *anti*? 1-F	major-**61**	0·0003
3-F	—	[a]	*syn* 2,3-$(OH)_2$, *syn*? 1-F	minor-**61**	0·0028
3-OMe	—	0·21	1-F, 3-OH mix (6 : 1)	**63**	0·028
3-Cl	—	[a]	1,1-F_2, 3-OH	**64**	0·037

to that adopted by **4**. The most active compound, **15**, a homologue of **11**, was reported to have an IC_{50} of <1 μM (equivalent to a K_i of <100 nM) and levels of herbicidal activity similar to those of **2** and **19**.[20]

In an initial investigation on the scope of linkage substitution, the hydroxyl group of **6** could neither be oxidised nor converted into a leaving group without olefin formation. The ketone **9**, reduced with borohydride to

TABLE 2—(*Continued*)





Substituent	*Compound No.*	K_i (μM)	*Substituent*	*Compound No.*	K_i (μM)
3-OH	—	40	3-OH: HET = 1,2,4-triazol-1-yl	**12**	0·47
2-OH	—	2900	2-OH: HET = 1,2,4-triazol-1-yl	**14**	7·0
			anti 3,4(OH)$_2$: HET = 1,2,4-triazol-3-yl	**35**	4·67
			syn 3,4(OH)$_2$: HET = 1,2,4-triazol-3-yl	**37**	0·235

[a] These compounds convert, at varying speeds, into **19** and eventually exhibit its K_i.

give an alternative synthesis of **2**, and the amine **10** were thus constructed *via* different strategies, also shown in Fig. 2. Whilst less inhibitory (K_i 15 nM) than **2**, the amine **10** was equally herbicidal, perhaps reflecting improved penetration and/or translocation due to reduced net negative charge. The ketone **9** was a much poorer inhibitor (K_i 1 μM), as were analogues containing a variety of other substituents at C-2, C-2 tertiary alcohols, and an olefin. The unsubstituted parent **deshydroxy-2** (K_i 72 nM) was unexpectedly active *in vitro* but was of little interest *in vivo*. The diethylaminosulfur trifluoride-derived fluoride (K_i 31 nM), which is prone to elimination, and the potentially propesticidal acetate were, in contrast, appreciably herbicidal.

Reductions in intrinsic activity of several orders of magnitude were noted when the phosphonate moiety was replaced by methyl phosphinate, carboxylate, sul-

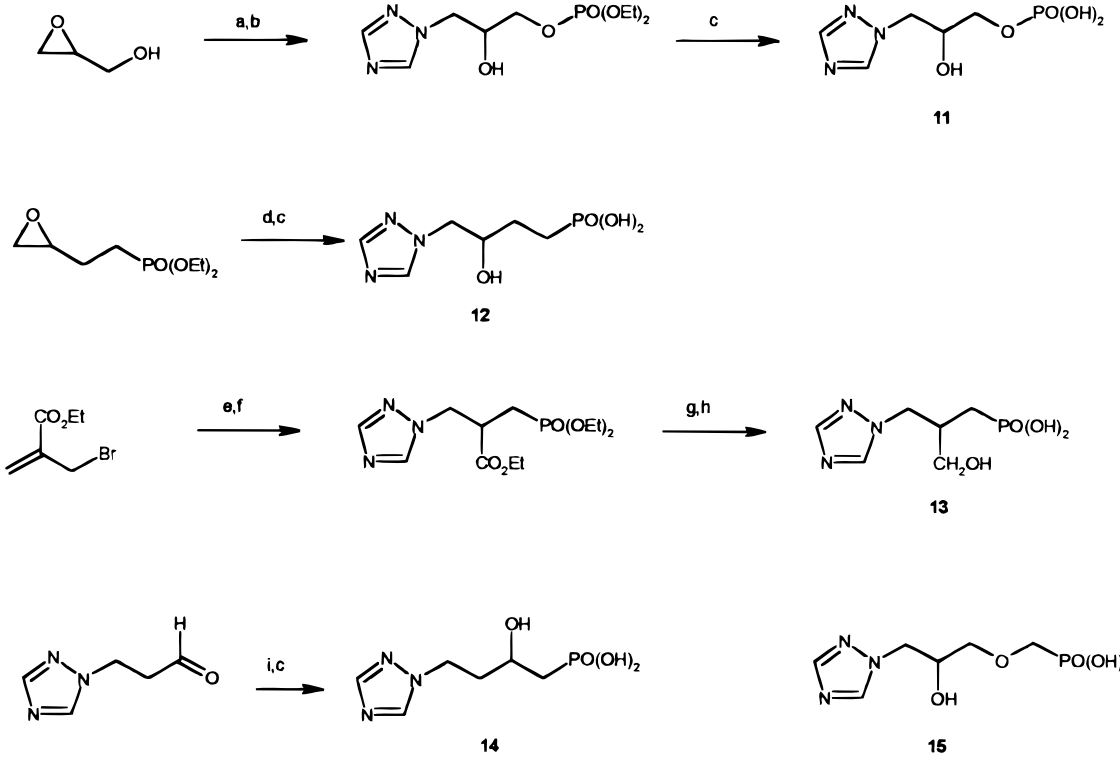


Fig. 3. 'Higher homologues': Syntheses of compounds **11**, **12**, **13** and **14**, and the structure of a literature example, compound **15**. (a) NaH, Et_2O, $ClPO(OEt)_2$ (b) 1,2,4-Triazole, CsF, EtOH, 20°, days (c) Me_3SiBr, CH_2Cl_2; MeOH; Amberlite CG-120 (d) 1-Trimethylsilyl-1,2,4-triazole, nBu_4NCl, 90° (e) $P(OEt)_3$ (f) 1,2,4-Triazole, CsF, MeCN (g) $NaBH_4$, *t*BuOH, MeOH (h) 12 M HCl; Amberlite CG-120 (i) $LiCH_2PO(OEt)_2$, THF.

phonate or boronate; syntheses are shown in Fig. 4. Some phosphonate half-esters, arising from basic hydrolysis of diesters, showed appreciable levels of herbicidal activity despite poor intrinsic activity. Chemical and/or metabolic conversion into the free acid was suspected.

Having established basic structure–activity relationships, the more challenging C-linked heterocycles were targeted. Not only did we expect triazoles to mimic **4**, rather than substrate **3**, but imidazoles could not be excluded as either inhibitors or alternative substrates. Furthermore, it is now possible to introduce a hydroxyl group adjacent to the heterocyclic ring since the products are no longer hemiaminals of suspect stability. The C-3 alcohol **19** was prepared by the two routes shown in Fig. 5, which have also been reported by workers at Ciba-Geigy.[18,23,24] Lithiation/formylation of 1-triphenylmethyl-1,2-4-triazole gave the expected sterically congested π-protected aldehyde **16**, in accord

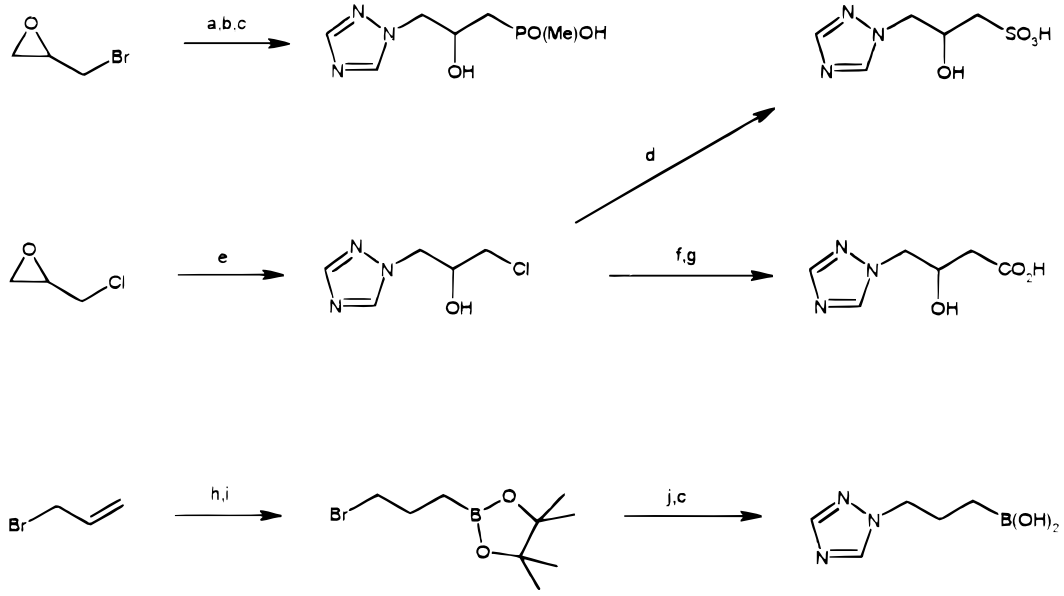


Fig. 4. Syntheses of the methylphosphinate, sulphonate, carboxylate and boronate analogues of the lead phosphonates. (a) $MeP(OEt)_2$ (b) 1,2,4-Triazole, K_2CO_3, EtOH (c) Me_3SiBr, CH_2Cl_2; H_2O (d) Na_2SO_3, H_2O (e) 1,2,4-Triazole, EtOH (f) NaCN, DMF (g) 6 M HCl (h) Catecholborane, neat (i) Pinacol, THF (j) 1,2,4-Triazole, K_2CO_3, MeCO*i*Pr.

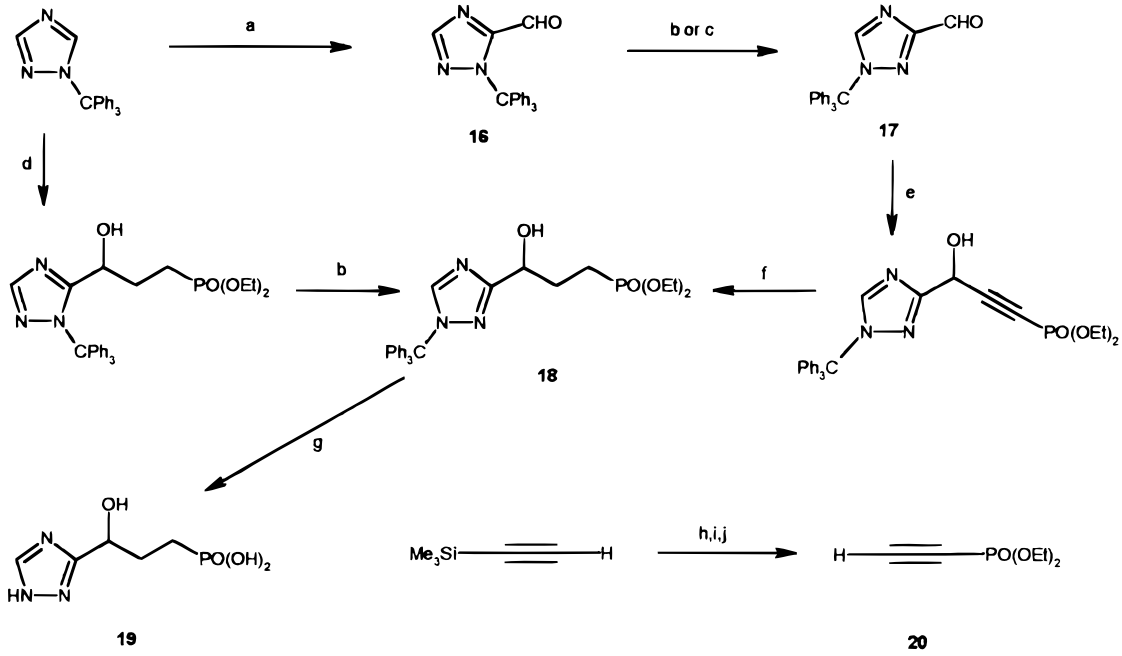


Fig. 5. Syntheses of the key intermediate **17**, for C-linked triazolylphosphonates, and the 3-hydroxypropylphosphonate, compound **19**. (a) *n*BuLi, $Me_2N(CH_2)_2NMe_2$, THF or $LiN(iPr)_2$, THF; DMF (b) CF_3CO_2H, CH_2Cl_2 (c) HCl gas, CH_2Cl_2 (d) *n*BuLi, $Me_2N(CH_2)_2NMe_2$, THF; $CHO(CH_2)_2PO(OEt)_2$; aq. NH_4Cl, $-70°$ (e) **20**, *n*BuLi, THF (f) H_2, PtO_2, *i*PrOH (g) Me_3SiBr, CH_2Cl_2, MeOH; Amberlite CG-120 (h) *n*BuLi, THF; $ClP(OEt)_2$ (i) $3\text{-}ClC_6H_4CO_3H$, CH_2Cl_2 (j) KF, EtOH.

with the lithiation chemistry of 1-benzyl-1,2-4-triazoles which has since been discussed.[31] In most cases, although not exclusively, the isomeric τ-tritylated series was preferred in downstream reactions. Whereas the aldehyde **16** was most conveniently converted into its isomer **17** by treatment with trifluoroacetic acid or with hydrogen chloride gas, triphenyl phosphine hydrobromide was often preferred for trityl group migrations at later stages. The acetylenic phosphonate **20** was prepared, as shown, avoiding hazardous chloroacetylenes. Whilst the alternative route appears to be more direct, the precise conditions of the triazole-aldehyde addition step were found to be critical, leading us to suspect that this reaction may be reversible. The hindered aldehyde **16** is not a useful substrate. The C-2 alcohol **24** was also prepared, as shown in Fig. 6, *via* a Mark rearrangement,[32] of a phosphite of the ynol **21**. 4-Dimethylaminopyridine was found to be an essential catalyst. Conversion of the allene **22** into the ketone **23** was best accomplished *via* an enol ether, rather than by direct hydrolysis. The conversion of **21** into **23**, *via* **22** and an enamine, has since been reported.[25] Further reduction and deprotection gave the C-2 alcohol **24**, more potent both as an inhibitor ($K_i < 1$ nM) and as a herbicide than the isomeric C-3 alcohol **19** (K_i 11 nM). The corresponding 4-imidazolyl analogues, made similarly, were expectedly poor inhibitors (K_i 40 μM, 2·9 mM respectively).

It was next queried whether the hydroxyl groups of the two potent inhibitors **19** and **24** interact with separate binding sites or, as it is possible adequately to superimpose the structures, only one. If two sites can be accessed simultaneously, the C2–C3 diol should be clearly superior to either alcohol. The *syn* diol **28** was prepared as shown in Fig. 7. The configuration of the Wittig product from the hindered aldehyde **16** was established to be almost exclusively *E*, by comparing the NMR spectrum of the derived *E*-phosphonate **27** with that of the corresponding *Z*-isomer **29**. Treatment of **25** with triphenylphosphine/bromine gave either the π- or the rearranged τ-protected bromide, depending upon the exact conditions of the reaction. Triphenylphosphine hydrobromide is believed to be the acidic catalyst responsible. The sterically hindered phosphonate **26** also underwent trityl group migration to give **27**, which was transformed *via* osmium-catalysed dihydroxylation into the *syn* diol **28**. It has since been reported that a modified Wittig reagent can be used to obtain a mixture containing a majority of the *Z*-olefin.[18] However, it is possible to obtain the *Z*-phosphonate **29** cleanly *via* Lindlar hydrogenation of the corresponding acetylene. The latter also underwent quantitative base-catalysed rearrangement, on basic alumina, to give the allene **22**, an intermediate in Fig. 6. Dihydroxylation and deprotection of **29** gave the *anti* diol **30**. The need for a more versatile intermediate led us to prepare the epoxide **31** *via* in-situ methyl trifluoromethyldioxirane oxidation of the *E*-olefin **27** as shown in Fig. 8. There is considerable discussion as to the exact nature of the oxidative species in in-situ ketone/Oxone® systems.[33] Ring cleavage of the epoxide **31** with water, under a variety of acidic conditions, was not stereospecific and presumably involved intermediates having significant carbocation character at C-3. In contrast the epoxide **33**, prepared from the free phosphonic acid **32** by treatment with preformed dimethyldioxirane, opened cleanly under basic conditions. Whilst 10% olefin remained, tungstate-catalysed oxidation of the crude product gave the *anti* diol **30**, containing no **28** as determined by NMR. The potential intermediacy of cyclic phosphonates, akin to **56** (Fig. 13), arising from intramolecular attack of the phosphonate dianion upon C-3, was not investigated. Whilst the spectra of **28** and **30** are all but identical, the complex methylene signals at δ 1·75–2·05 (2H, m) are differentially shifted upfield in their respective sodium

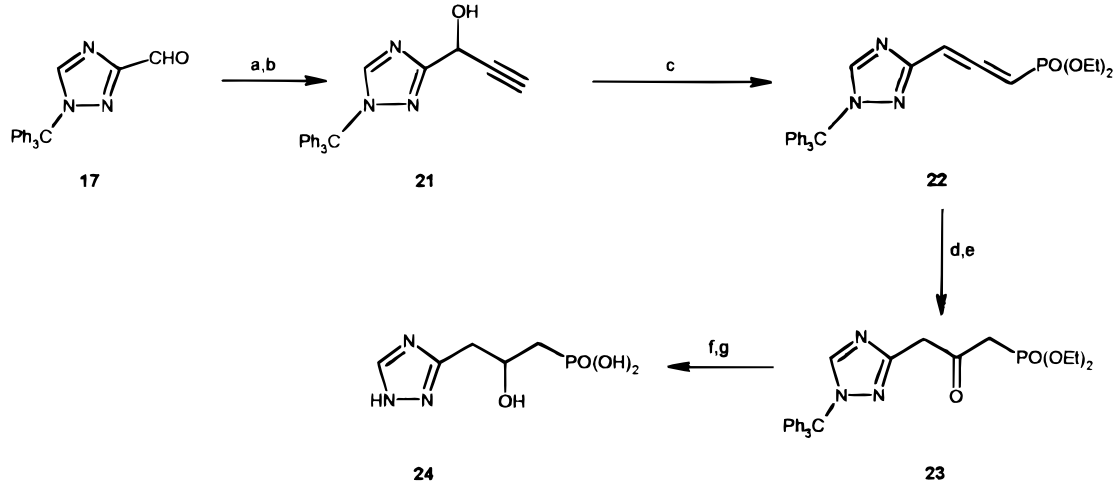


Fig. 6. Synthesis of the 2-hydroxyphosphonate, compound **24**. (a) $Me_3SiC{\equiv}CH$, *n*BuLi, THF (b) KF, MeOH (c) $ClP(OEt)_2$, Et_3N, 4-dimethylaminopyridine, CH_2Cl_2 (d) NaOEt, EtOH (e) 12 M HCl, rapid (f) $NaBH_4$, MeOH (g) Me_3SiBr, CH_2Cl_2; H_2O; Amberlite CG-120.

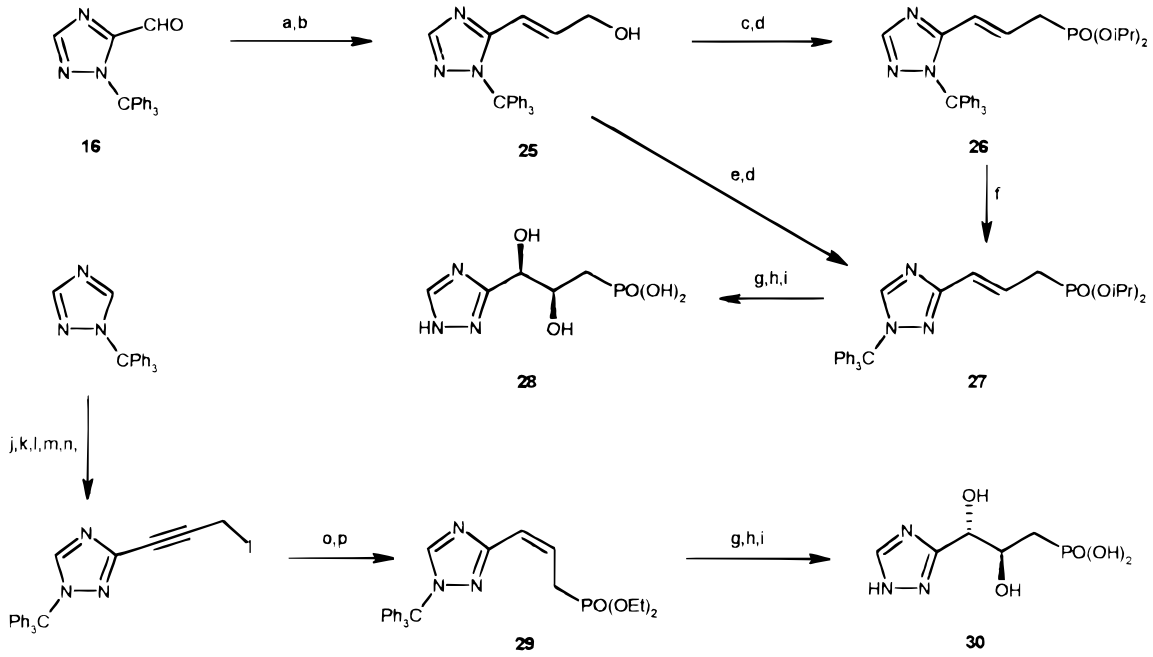


Fig. 7. Syntheses of the *syn*-2,3-dihydroxypropylphosphonate, compound **28**, and its *anti* diastereoisomer, compound **30**. (a) $Ph_3P{=}CHCHO$, EtOAc (b) $NaBH_4$, *i*PrOH, $CHCl_3$ (c) PPh_3, $CHCl_3$; CBr_4 added slowly, 0° (d) $P(OiPr)_3$ (e) Ph_3P, Br_2, $CHCl_3$; often $PPh_3.HBr$, $CHCl_3$ (f) $PPh_3.HBr$, $CHCl_3$ (g) OsO_4, *N*-Methylmorpholine *N*-oxide, *t*BuOH, THF, H_2O (h) CH_3CO_2H, H_2O, THF (i) Me_3SiBr, CH_2Cl_2 ; MeOH; Amberlite CG-120 (j) *n*BuLi, $Me_2N(CH_2)_2NMe_2$, THF; I_2 (k) $PPh_3.HBr$, CH_2Cl_2 (l) $HC{\equiv}CCH_2OH$, $(Ph_3P)_4Pd$, CuI, Et_3N, DMF (m) $PPh_3.Br_2$, Et_3N, CH_2Cl_2 (n) NaI, Me_2CO (o) $(EtO)_2POSiMe_3$, CH_2Cl_2 (p) H_2, *i*PrOH, Lindlar catalyst.

salts: δ 1·0 (1H, m), 1·2 (1H, m) for **28** and δ 1·4 (1H, m), 1·7 (1H, m) for **30**. Similar behaviour was noted for other *syn-anti* pairs. The *syn* diol **28** proved to be considerably more potent, both as an inhibitor (K_i 1·8 nM) and as a herbicide, than the *anti* diol **30** (K_i 253 nM). However neither is more potent than either of the analogues containing only one hydroxyl group, in particular the C-2 alcohol **24** ($K_i < 1$ nM), implying that there may indeed be only one binding site on the enzyme in this region. Extremely similar results for **28** and **30** have been obtained, and comparable conclusions drawn, by workers at Ciba-Geigy using the wheat germ enzyme.[18]

At this stage, confirmation was sought that a three-carbon spacer remained optimum for C-linked heterocycles. Homologues of the diols **28** and **30**, i.e. **37** and **35** respectively, were synthesised as shown in Fig. 9. The

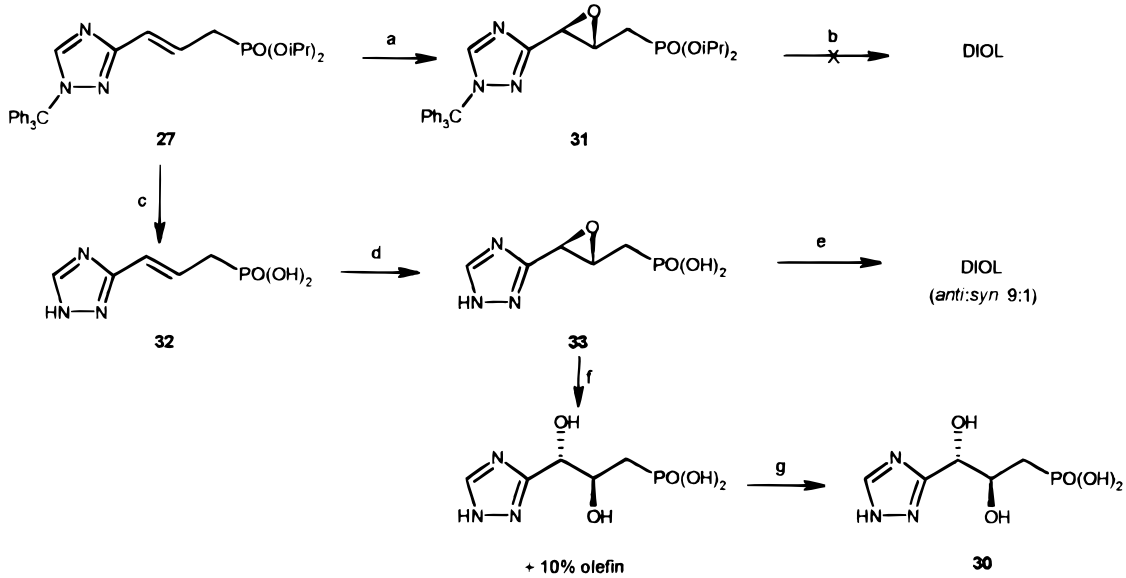


Fig. 8. An alternative synthetic route to the *anti* 2,3-dihydroxypropylphosphonate, compound **30**. (a) Oxone®, CF_3COMe, CH_2Cl_2, 18-Crown-6, H_2O, pH 7·4 (b) Various hydrolytic conditions (c) Me_3SiBr, CH_2Cl_2 ; H_2O; Amberlite CG-120 (d) Dimethyldioxirane, H_2O, Me_2CO, (e) H_2O (f) NaOH, H_2O (g) Na_2WO_4, H_2O_2, H_2O; Amberlite CG-120.

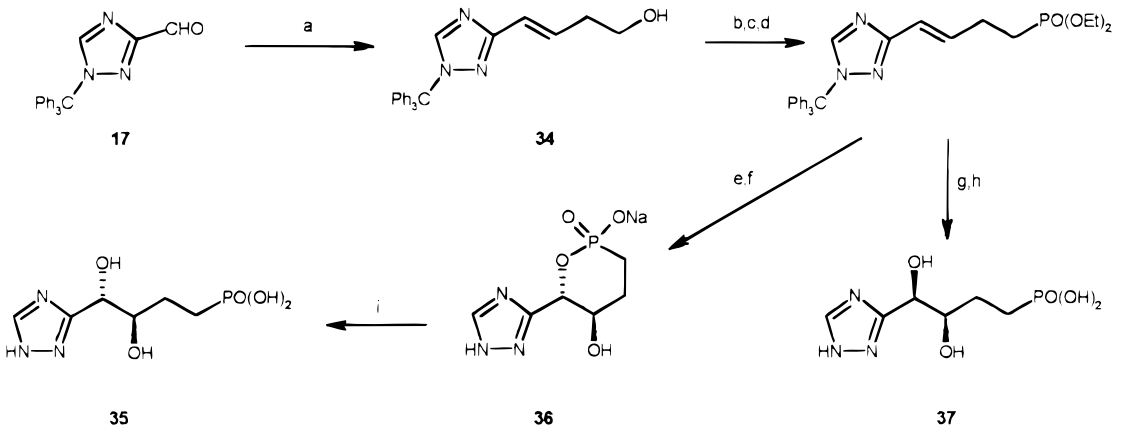


Fig. 9. Syntheses of the *anti* 3,4,-dihydroxybutylphosphonate, compound **35**, and its *syn* diastereoisomer, compound **37**. (a) $Ph_3P^+(CH_2)_3OH\ Br^-$, K_2CO_3, *i*PrOH (b) $MeSO_2Cl$, Et_3N, CH_2Cl_2 (c) NaI, Me_2CO, (d) $P(OEt)_3$ (e) Dimethyldioxirane, Me_2CO, H_2O (f) NaOH, H_2O; Dowex CCR-2 to pH 7 (g) OsO_4, *N*-Methylmorpholine *N*-oxide, *t*BuOH, THF, H_2O (h) Me_3SiBr, CH_2Cl_2 ; MeOH; Amberlite CG-120 (i) 2 M HCl, reflux; Amberlite CG-120.

Wittig olefination was now not selective (60 : 40) and only the major *E*-isomer **34**, as established by NMR, was progressed. Introduction of the diastereoisomeric vicinal diol functionalities paralleled that used for the lower homologues. However, epoxide cleavage under basic conditions gave rise to an isolated intermediate, the six-membered cyclic phosphonate **36**. As expected,[34] further drastic hydrolysis was now necessary to release the diol **35**. Both the latter (K_i 4·6 μM) and its *syn* diastereoisomer **37** (K_i 235 nM) were considerably (18 ×, 130 ×) less active than the corresponding lower homologues.

The similar weed control shown by **2** and **10**, despite a 25-fold difference in intrinsic activity suggested potential in-vivo advantages of amines over alcohols. This prompted us to investigate whether net charge might be important for penetration and/or translocation. The four possible regio- and stereo- isomeric amino alcohols **39** and **41** (Fig. 10), **45** and **46** (Fig. 11), the *syn* and *anti* diamines **47** and **48** (Fig. 11), and the regioisomeric monoamines **52** and **53** (Fig. 12) were targeted.

Syntheses of the *anti* and *syn* 3-amino-2-ols **39** and **41** are shown in Fig. 10. The epoxide **31** was attacked by azide ion, in the presence of ammonium chloride, at the 'benzylic' C-3 with inversion of configuration to give only the *anti* azido-alcohol **38**. Further deprotection and reduction gave **39**. A Sharpless osmium-catalysed oxyamination[35] of the *E*-olefin **27** also proceeded to give a single isomer, the *syn* hydroxy carbamate **40**, the regiochemistry of which was confirmed only after extensive spectroscopic investigation. Global deprotection with trimethylsilyl bromide gave **41**. The considerably greater potency of the latter (K_i 8 nM) compared with its *anti* diastereoisomer **39** (K_i 75 nM) was not unexpected, given the values for the respective diols. Furthermore, the increase resulting from the introduction of an amino group at C-3 was later mirrored in the 3-amine **53** (Fig. 12) itself. Both were potent herbicides, reflecting the increased in-vivo activity of amines, with **41**, more active than **39**, being as effective as **2**.

The regioisomeric 2-amino-3-ols **45** and **46** were also approached *via* the azido alcohol **38** as shown in Fig.

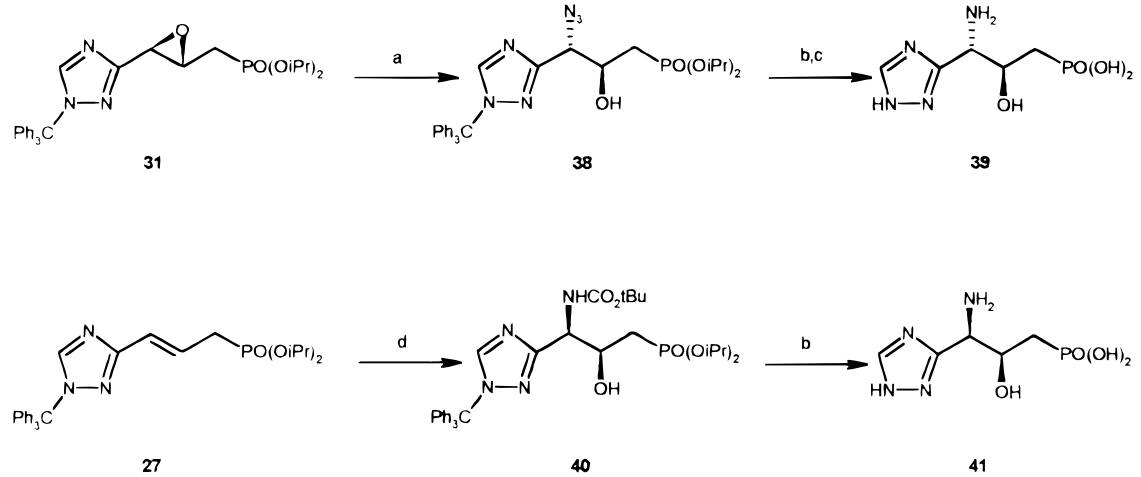


Fig. 10. Syntheses of the *anti* 3-amino-2-hydroxypropylphosphonate, compound **39**, and its *syn* diastereoisomer, compound **41**. (a) NaN_3, NH_4Cl, H_2O, MeOH (b) Me_3SiBr, CH_2Cl_2 ; MeOH; Amberlite CG-120 (c) H_2, PtO_2, H_2O (d) *t*BuOCONClNa (ex *t*BuOCONH$_2$, *t*BuOCl, NaOH, MeOH), $AgNO_3$, OsO_4, MeCN, H_2O.

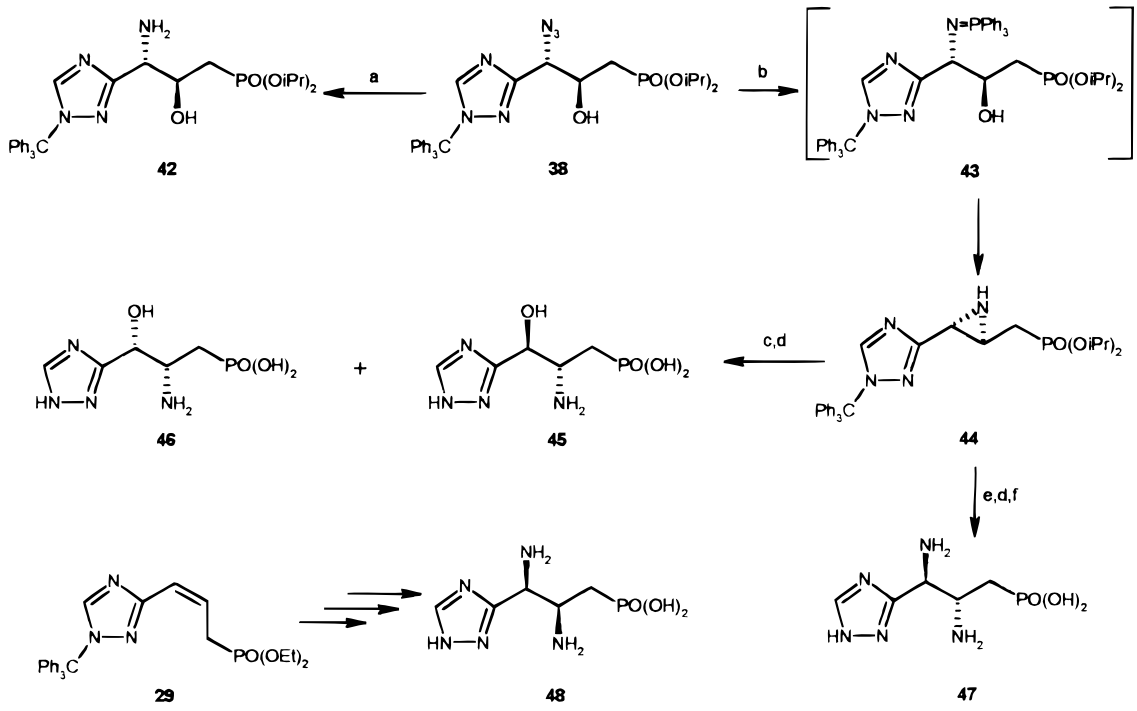


Fig. 11. Syntheses of the *anti* 2-amino-3-hydroxypropylphosphonate, compound **45**, its *syn* diastereoisomer, compound **46**, and the *anti* 2,3-diaminopropylphosphonate, compound **47**. (a) PPh_3, THF; H_2O (b) PPh_3, THF; $PPh_3.HBr$; H_2O (c) $tBuOCO_2NC(CN)Ph$, Et_3N, dioxan, H_2O (d) Me_3SiBr, CH_2Cl_2; MeOH; Amberlite CG-120 (e) NaN_3, NH_4Cl, MeOH, H_2O (f) H_2, PtO_2, H_2O.

11. Literature precedents suggested that its treatment with triphenylphosphine should give the aziridine **44** (see, for example, Ref. 36). However, whilst the reaction is solvent-dependent the major, usually sole, product was merely that of reduction, the 3-amino-2-ol **42**. It is necessary for the putative intermediate, the iminophosphorane **43**, to undergo transfer of phosphorus from nitrogen to oxygen. The addition of triphenylphosphine hydrobromide, to protonate nitrogen and thus weaken its bond to phosphorus, led to the formation of the required aziridine **44** in high yield. This proved to be remarkably resistant to attack by oxygen nucleophiles without further degradation. Traditional hydrolytic cleavage, employing dilute perchloric acid, merely promoted detritylation. Activation of ring scission was achieved *via* carbamoylation. Silicon-mediated phosphonate deprotection was accompanied by ring opening to give the desired amino alcohols **45** and **46**, free of all protection because a *tert*-butyl carbamate was employed. Initial formation of a 1 : 1 *syn* : *anti* mixture might suggest the involvement of an intermediate carbonium ion at C-3. Surprisingly, purification on a

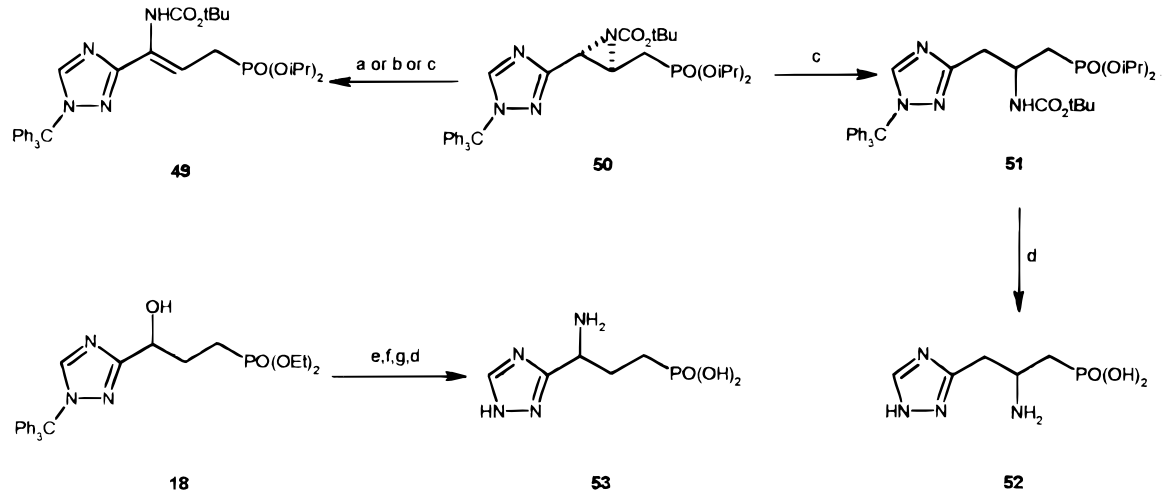


Fig. 12. Syntheses of the 2-aminopropylphosphonate, compound **52** and its 3-amino regioisomer, compound **53**. (a) $NaNH_2$, 9,10-Dihydroanthracene, THF (b) $LiBHEt_3$, THF (c) $LiBHEt_3$, Me_3SiCl, THF (d) Me_3SiBr, CH_2Cl_2; MeOH; Amberlite CG-120 (e) Br_2, nBu_3P, Et_3N, CH_2Cl_2 (f) NaN_3, 15-Crown-5, DMF, H_2O (g) H_2, 10% Pd/C, *i*PrOH.

strong acid resin appeared to result in extensive epimerisation to give a 9 : 1 *anti* : *syn* ratio. Intermediate 1,2-oxaphospholanes, akin to **56** (Fig. 13), may be implicated. The *in vitro* activity (K_i 580 nM) of the latter mixture was in line with that of the *anti* diol **30**, but in striking contrast to that of its regioisomer **39** (K_i 75 nM). Levels of herbicidal activity were also much lower.

The diamines **47** and **48**, having net negative charge reduced still further, were also approached as shown in Fig. 11. The aziridine **44** underwent cleavage with azide ion, albeit reluctantly, to give only the *anti* 3-azido-2-amine. The derived *anti* diamine **47** exhibited only moderate activity (K_i 580 nM), identical to that of the *anti* amino alcohol **45**. Herbicidal activity was, however, considerably higher, suggesting that the further reduction in net charge may be beneficial. At the time, there were no known methods of metal-catalysed diamination by which to convert the *E*-olefin **27** into the outstanding *syn* diamine and this situation still pertains. However, it was expected that the *Z*-olefin **29** could be converted, *via* steps analogous to those used for the *E*-olefin, *via* an aziridine into the *syn* diamine **48**. Unfortunately, this route was never progressed beyond the epoxide.

The remaining targets of this phase of the programme were the C-2 and C-3 amines (**52** and **53**), the syntheses

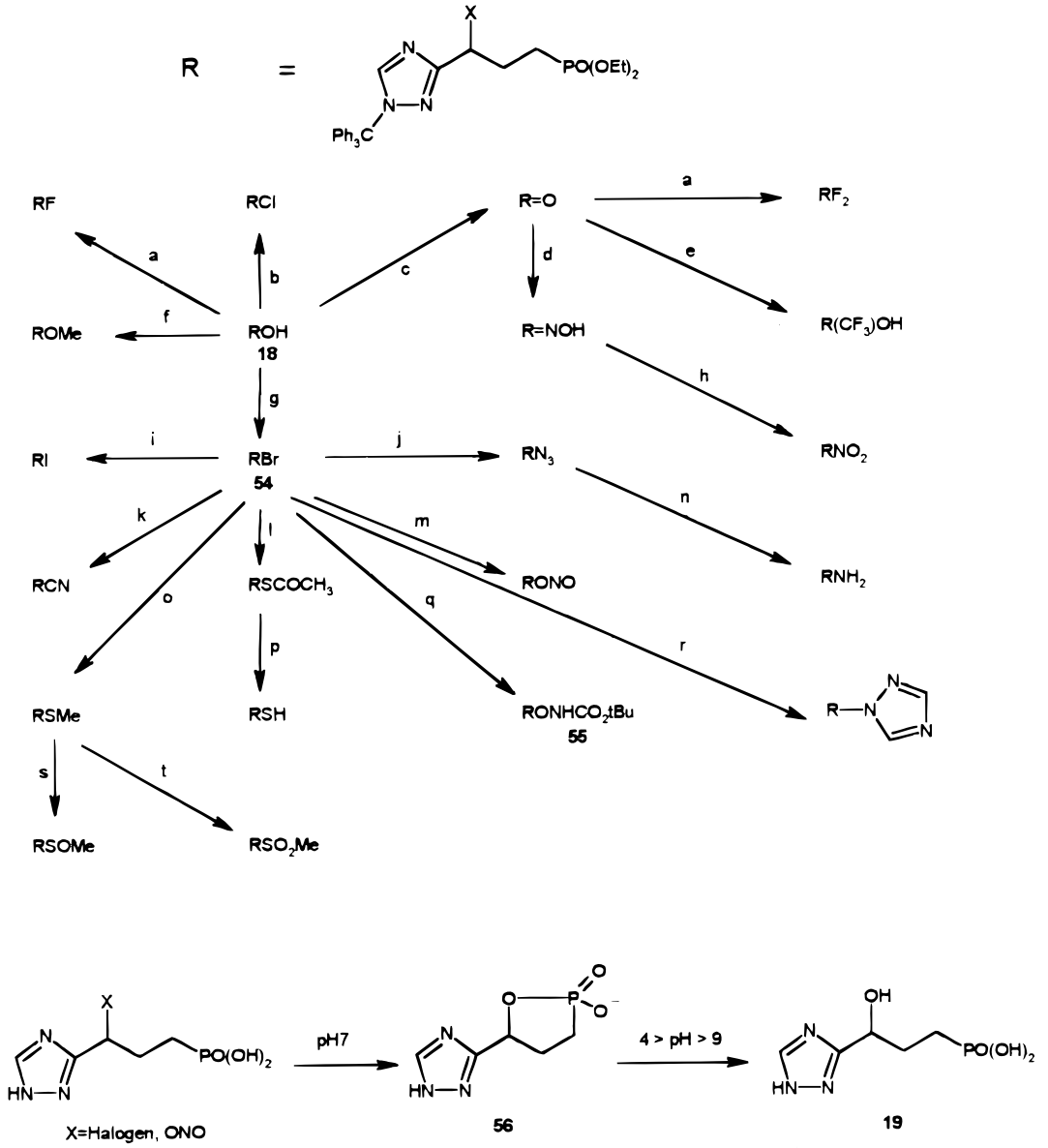


Fig. 13. A compendium of synthetic routes to 3-substituted propylphosphonates and the facile hydrolysis which some examples undergo. (a) Et_2NSF_3 (b) $SOCl_2$, pyridine (c) $(COCl)_2$, Me_2SO, CH_2Cl_2 ; Et_3N (d) $NH_2OH.HCl$, pyridine, EtOH (e) Me_3SiCF_3, nBu_4NF, THF (f) NaH, MeI, 15-Crown-5, THF (g) Br_2, nBu_3P, Et_3N, CH_2Cl_2 (h) H_2O_2-urea, $(CF_3CO)_2O$, MeCN (i) NaI, Me_2CO (j) NaN_3, 15-Crown-5, H_2O, DMF (k) NaCN, 15-Crown-5, DMF (l) KSAc, 18-Crown-6, THF (m) $AgNO_2$, Et_2O (n) H_2, 10% Pd/C, *i*PrOH (o) NaSMe, EtOH (p) NaOMe, MeOH (q) *t*BuOCONHOH, KOH, EtOH (r) NaH, 1,2,4-triazole, 15-Crown-5, DMF (s) $NaIO_4$, H_2O, MeOH (t) 3-Cl-$C_6H_4CO_3H$, CH_2Cl_2.

of which are shown in Fig. 12. Whilst the hydroxyl substituent, in the C-2 alcohol derived from reduction of **23** according to Fig. 6, might be converted into a leaving group, displacement with a nitrogen nucleophile would be expected to be accompanied by extensive elimination. Not only would cleavage of the aziridine **50**, prepared as shown in Fig. 11, be expected to proceed in the desired regiochemical sense but activated aziridines are known to be reduced with 9,10-dihydro-9-sodioanthracene.[37] Unfortunately, the acidity of the protons adjacent to phosphorus is such that the sole product was **49**, resulting from base-catalysed rearrangement. Lithium triethylborohydride gave similar results until its basicity was attenuated by the addition of 20 mol% of trimethylsilyl chloride. The desired carbamate **51** then formed, albeit in only poor yield, was deprotected to give the 2-amine **52**. The isomeric 3-amine **53** was prepared from the corresponding alcohol **18**, as shown in Fig. 12. Once again, replacement of hydroxy by amino at C-3 resulted in a slight improvement in activity (K_i 11 nM, 9·5 nM), whilst that at C-2 gave a marked decrease ($K_i < 1$ nM, 24 nM). As a herbicide, the activity of **53** approached that of **2** but **52** was considerably less active.

The parent unsubstituted C-linked triazole, synthesised from the allylic alcohol **25** *via* hydrogenation, bromination, Michaelis–Becker introduction of phosphorus and deprotection, is only a very weak inhibitor (K_i 9·6 μM) compared with the corresponding *N*-linked triazole (K_i 72 nM). However, the introduction of a single hydroxyl group is much (*c*.80×) more advantageous than it is in the latter series. Furthermore, it has been shown that the effect of introducing such groups depends upon their relative stereochemistry and can be deleterious, leading to the suggestion that the enzyme may have only one binding site in this region. The problems of promoting substitution over elimination at C-2 led us to study the effects of alternative substituents, some of which are shown in Fig. 13 and yet more included in a published patent,[21] at C-3 in isolation. The alcohol **18** (see also Fig. 5) and the derived bromide **54** were used as pivotal intermediates as shown. Phosphonate esters were generally converted into the target acids by treatment with trimethylsilyl bromide, followed by water or methanol, and chromatography on a strong acid resin. The *tert*-butyloxycarbonyl protection in **55** was also removed during this process. The C-3 iodide required trimethylsilyl iodide deprotection to prevent formation of the corresponding C-3 bromide. Apart from the alcohol **19** and the amine **53**, previously discussed, the free acids were only weakly inhibitory yet, surprisingly, a number were appreciably herbicidal. This apparent anomaly was resolved when it was established that the compounds were mostly those which, whilst essentially stable at pH 2, are converted into a common degradation product at physiological pH. Although the chloride, bromide and nitrite were transformed in phosphate buffer within an hour, the fluoride required several weeks. Extensive NMR evidence, in particular a 20 ppm downfield shift in the ^{31}P resonance, identified the product as the salt **56** of a 1,2-oxaphospholane.[34] Correlation of the time-course of chemical changes with enzyme inhibition suggests that this material is not itself appreciably inhibitory ($K_i > 500$ nM), but that it is converted into the alcohol **19**. Chemical hydrolysis is slow at pH 7 but rapid below pH 4 or above pH 9. In-vivo activity in excess of that of **19** presumably results from enhanced penetration and/or translocation, either of material applied or of the intermediate **56**. Herbicidal activity may also derive from metabolic activation. The herbicidal C-3 acetate, for example, is not converted into the cyclic phosphonate *in vitro*, but the fate of the moderately active azide is not known.

It is generally recognised that mono- and difluorinated phosphonates are more satisfactory isosteric and isopolar replacements for naturally occurring phosphates than the parent phosphonates.[3,38–41] This may be due merely to the reduction in the second pKa, thereby increasing the proportion of dianion at physiological pH, but more subtle effects have been discussed.[42,43] Carbanions adjacent to phosphonate are known to be fluorinated by *N*-fluorodibenzenesulphonamide,[44] but we were unable to find conditions under which the allylic phosphonate **27** (Fig. 7) gave more than a trace of the desired 1- or 1,1-fluorinated products; multicomponent mixtures contained fluorine at both C-1 and C-3. Alternatively, fluorine can be introduced as an anion, as shown in Fig. 14. Thus the allylic bromide **57** was displaced with fluoride ion and bromine reintroduced under free-radical conditions. The resultant **59** underwent an Arbusov reaction to give the fluorophosphonate **60**, but attack by phosphorus at bromine, effectively a reduction regenerating **58**, was also observed. Further osmium-catalysed dihydroxylation gave a 3 : 1 mixture of diastereoisomers **61**, separable at the ester level. The relative stereochemistry of fluorine could not be determined by spectroscopic techniques. However, both Kishi's empirical rule on the stereochemistry of osmium tetroxide oxidations of allylic alcohols[45] and observations on the stereoselective epoxidation of allylic fluorides[46] would favour the *syn-anti* configuration. Introduction of fluorine affected binding to IGPD only marginally (K_i of major 0·3 nM, minor 2·8 nM versus the parent 1·8 nM). Herbicidal activity was somewhat reduced. The stepwise introduction of fluorine into the C-3 alcohol **19** was achieved using electrophilic fluorination[44] of the silyl ether **62**. Inhibition constants for both the monofluorinated **63** (K_i 28 nM), obtained as an inseparable mixture of diastereoisomers in a ratio of 6 : 1, and the difluorinated **64** (K_i 37 nM) were similar to that of the unfluorinated parent **19** (K_i 11 nM). In contrast, whilst **63** was at least as active as its parent *in vivo*, **64** was only poorly active. This could reflect its increased net charge at physiologi-

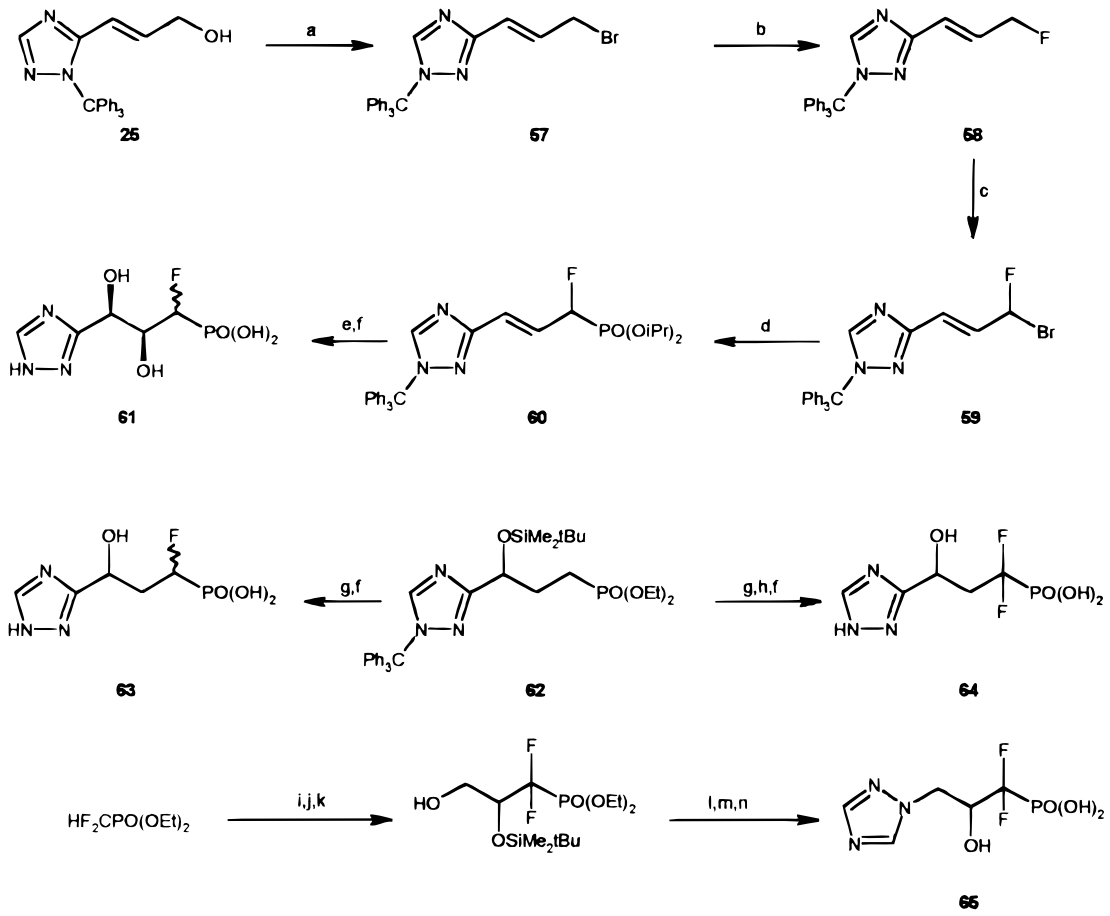


Fig. 14. Syntheses of α-fluorophosphonates, compounds **61** and **63**, and α,α-difluorophosphonates, compounds **64** and **65**. (a) PPh_3, Br_2, $CHCl_3$, 25°, hours, often PPh_3.HBr (b) nBu$_4$NF, THF (c) *N*-Bromosuccinimide, azobisisobutyronitrile, CCl_4 (d) P(O*i*Pr)$_3$ (e) OsO_4, *N*-Methylmorpholine *N*-oxide, *t*BuOH, THF, H_2O (f) Me_3SiBr, CH_2Cl_2; H_2O; Amberlite CG-120 (g) LiN(*i*Pr)$_2$, $(PhSO_2)_2NF$, THF, −78° (h) LiN(*i*Pr)$_2$, $(PhSO_2)_2NF$, THF, −100° (i) LiN(*i*Pr)$_2$, THF; $PhCH_2OCH_2CHO$ (j) *t*BuMe$_2$SiOSO$_2$CF$_3$, 2,6-lutidine, CH_2Cl_2 (k) H_2, Pd(OH)$_2$/C, 4-Me-$C_6H_4SO_3H$, EtOH (l) $(CF_3SO_2)_2O$, pyridine (m) 1,2,4-Triazole, NaH, DMF (n) Me_3SiBr, CH_2Cl_2; H_2O; Amberlite CG-120.

cal pH. However, the introduction of two fluorine atoms into our N-linked triazole **2** to give **65**, also shown in Fig. 14, resulted in somewhat decreased inhibition (K_i 8 nM versus 0·6 nM) but accompanied by only a marginal reduction in herbicidal activity! After the previous close correspondence between activities *in vitro* and *in vivo*, our assay using the recombinant yeast enzyme now seemed to be a poor predictor for fluorophosphonates. The anomaly might stem from subtle variations in this region between yeast and plant enzymes, but differential uptake, translocation or metabolism could also be responsible.

4 CONCLUSIONS

It has been demonstrated that inhibition of imidazoleglycerol phosphate dehydratase constitutes a valid target for potential herbicides. Potent active-site inhibitors, some having K_i values of less than 1 nM, show herbicidal effects akin to those of glyphosate. Application rates are very similar, although species sensitivity is, expectedly, not identical. Whilst no direct comparison can be made over the whole range of analogues, Compounds Nos **2**, **10**, **24**, **28**, **41**, **53**, and several of those converted into **19**, *in vitro* or *in vivo*, are amongst the most active. It is difficult to imagine cost-efficacy advantages for the C-linked, over the N-linked, series. Exploration of the location of potential binding sites has identified three important regions, the heterocycle, the phosphonate, and a polar substituent borne on the linking group. Further logical progress awaits a high-resolution X-ray structure determination of an enzyme-inhibitor complex.

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